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Aquatic animal behaviour test – video system tracks fish movements

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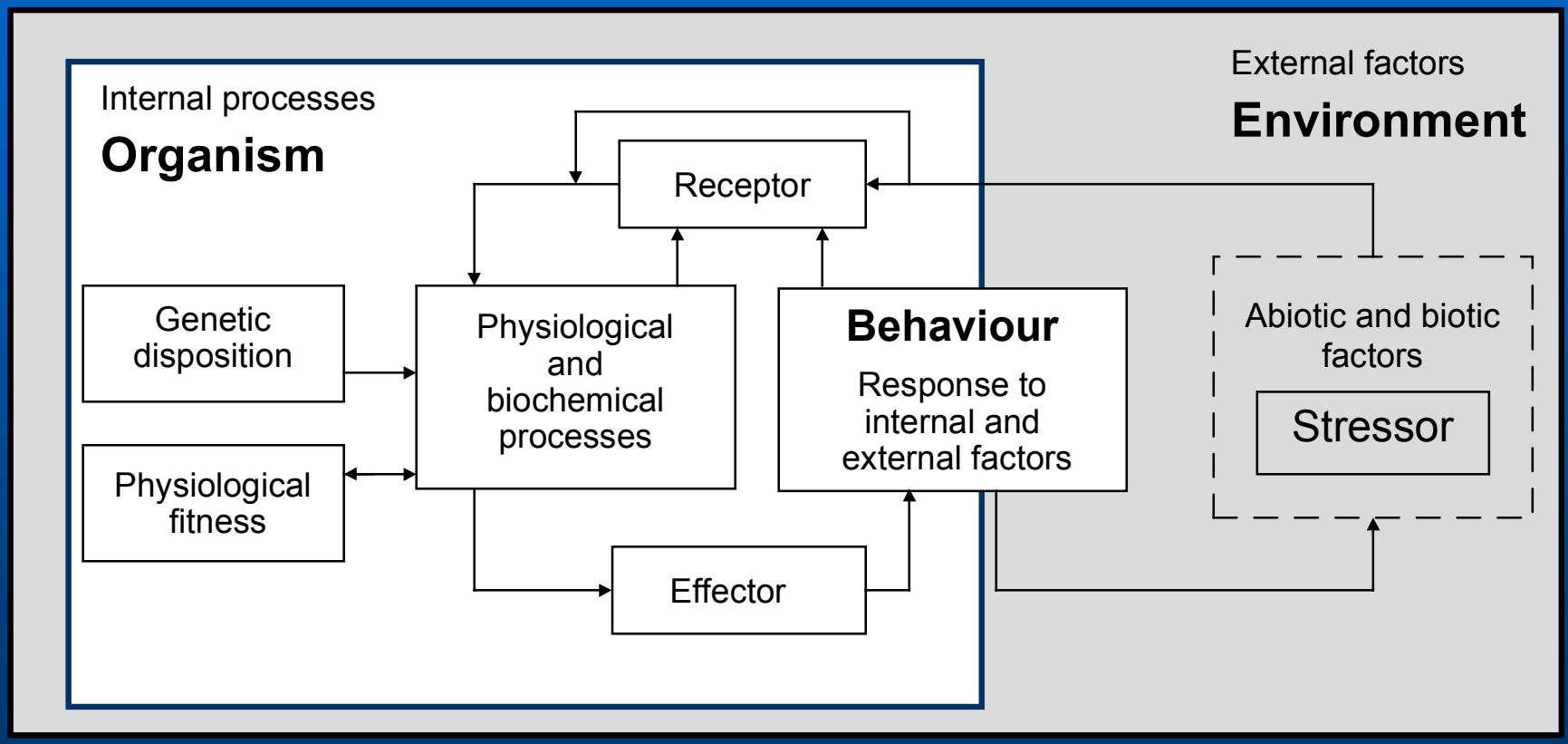
Topics

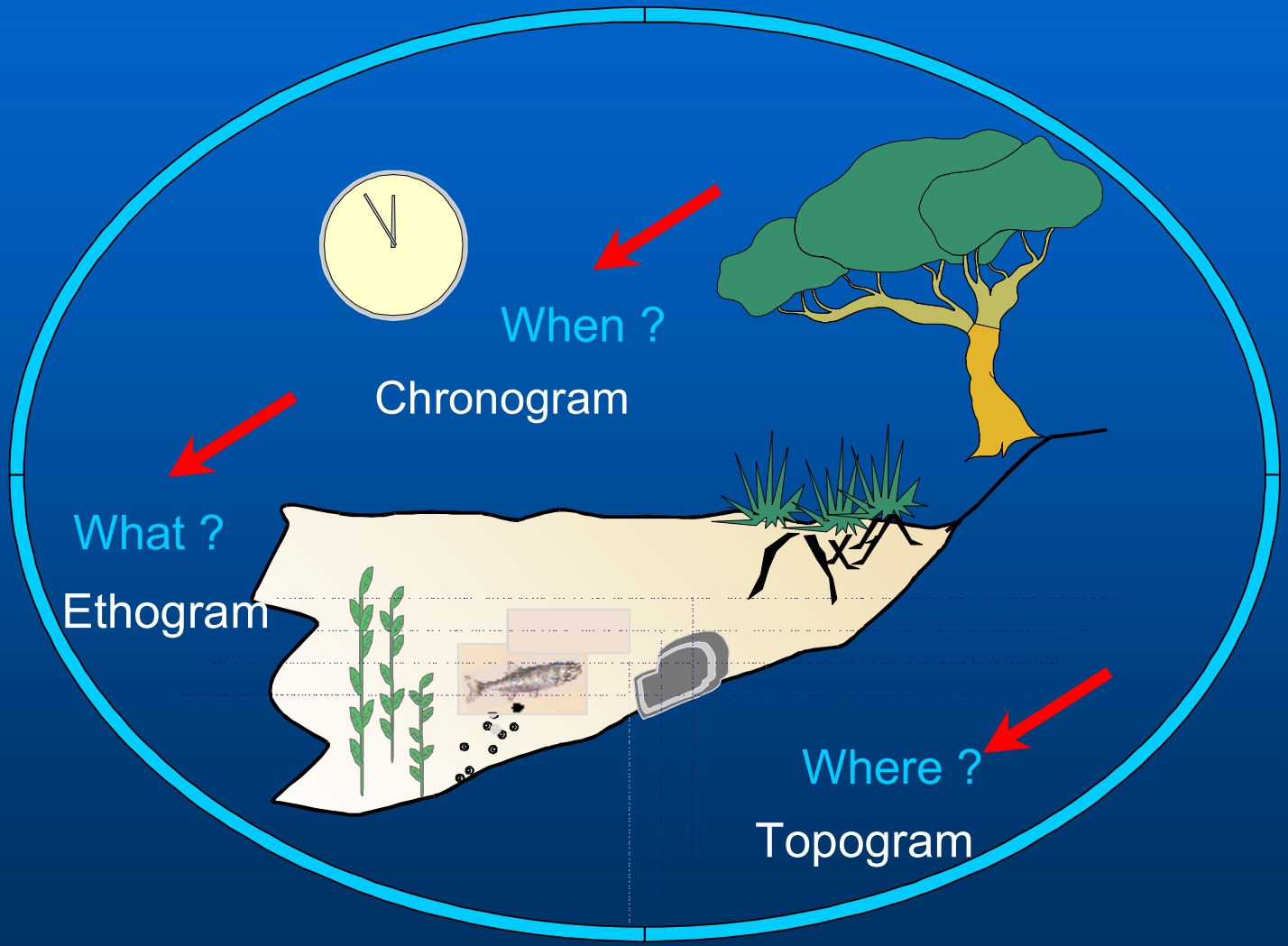
- Principles and methods of automated animal behaviour testing
  - *Behaviour of fish and what does it tell us*
  - *Method of video analysis of fish behaviour – the example of BehavioQuant*
- Selected results of behavioural video analysis investigating the influence of toxic substances
  - *Experimental set-up*
  - *Influences of MC-LR on general activity parameters*
  - *Analysis and significance of activity rhythms*
  - *Comparison of PCB 28 influences to MC-LR*

Behaviour

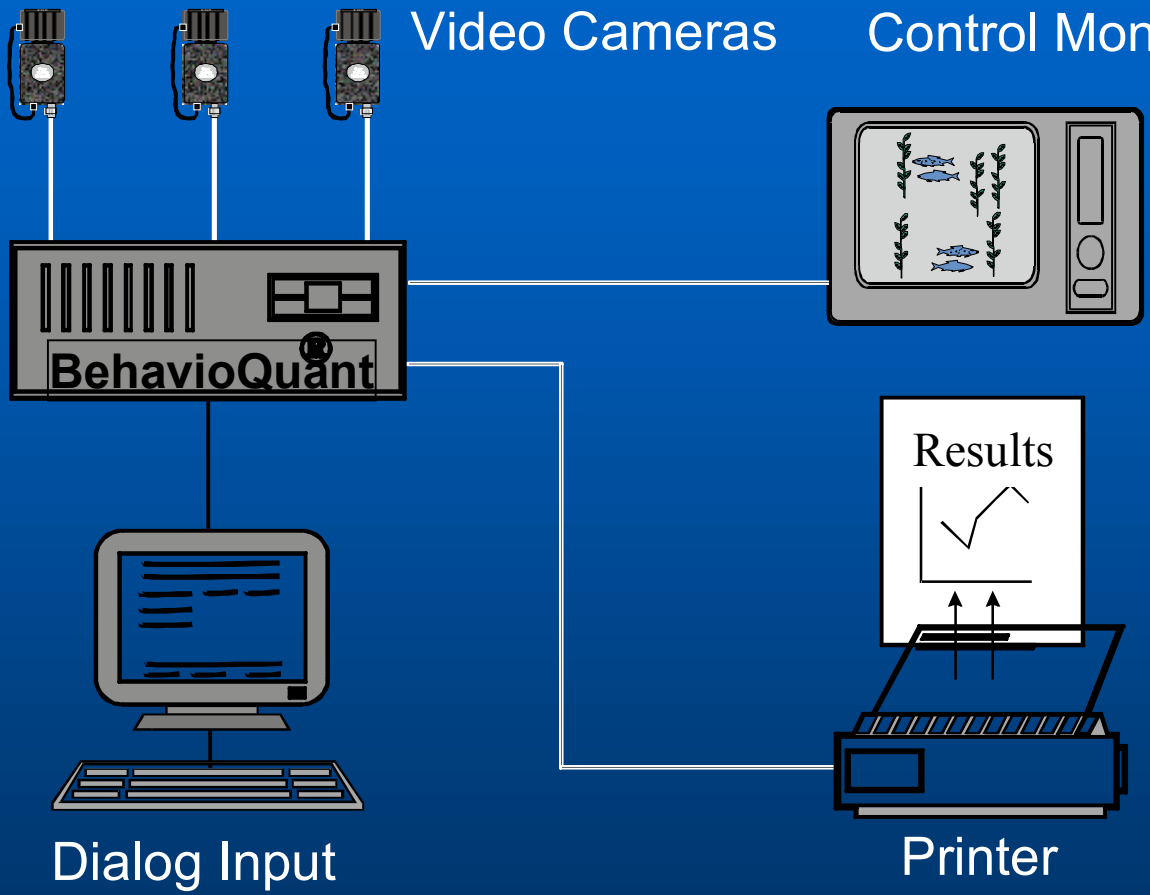


Baganz, 2005

Recording of fish behaviour

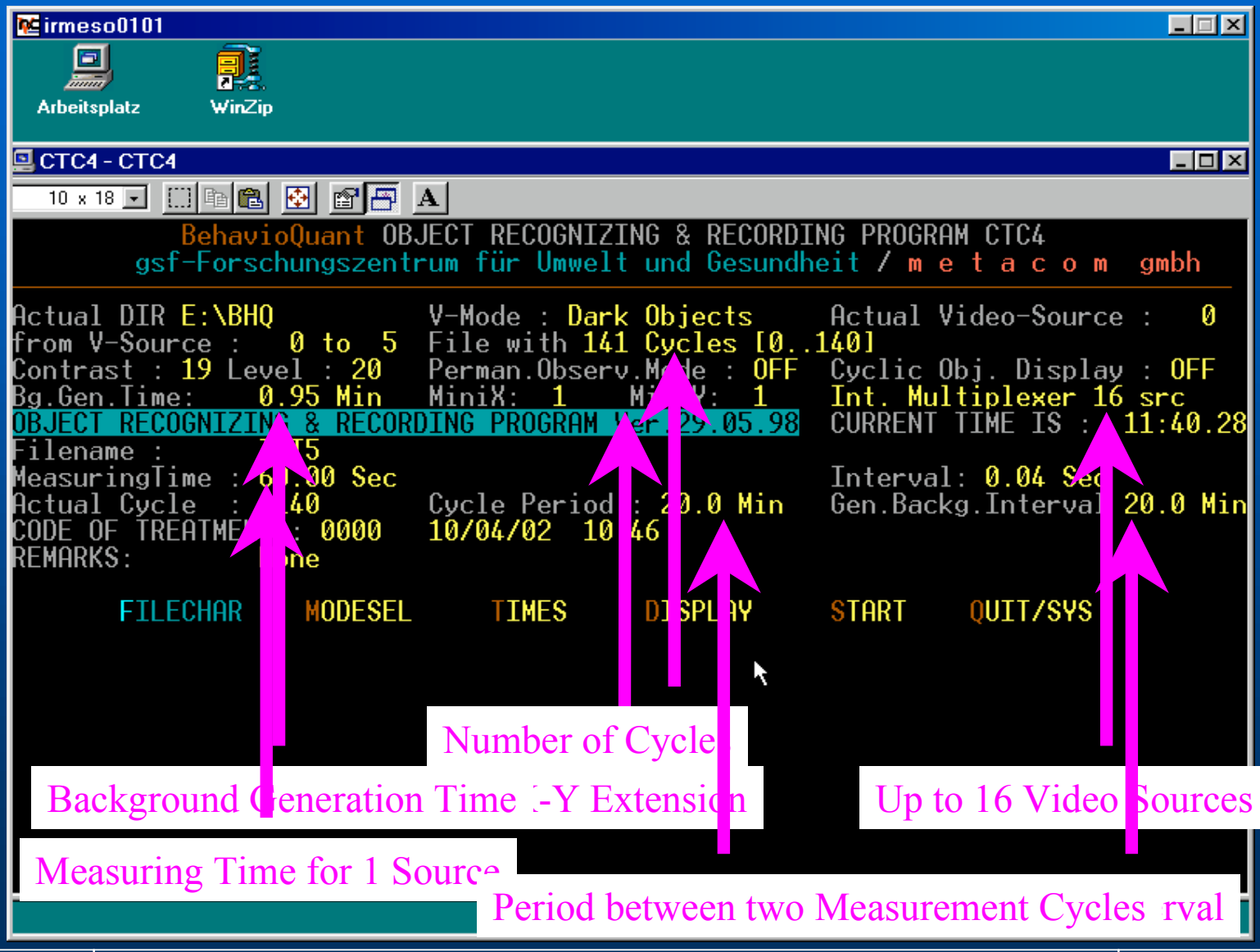


Functional principle

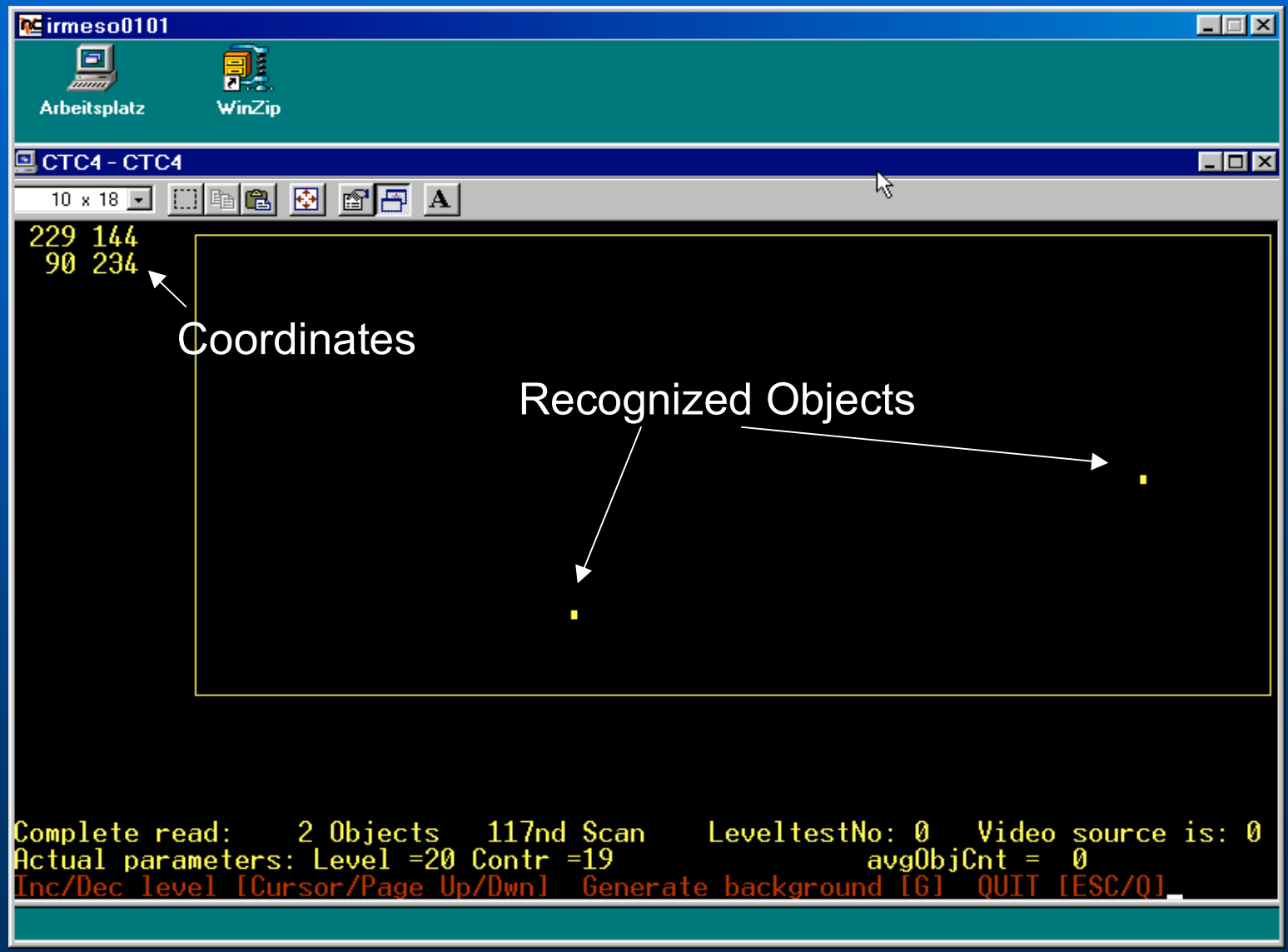


BehavioQuant®-System (from Lorenz, 1993)

BehavioQuant - software and abilities (1)



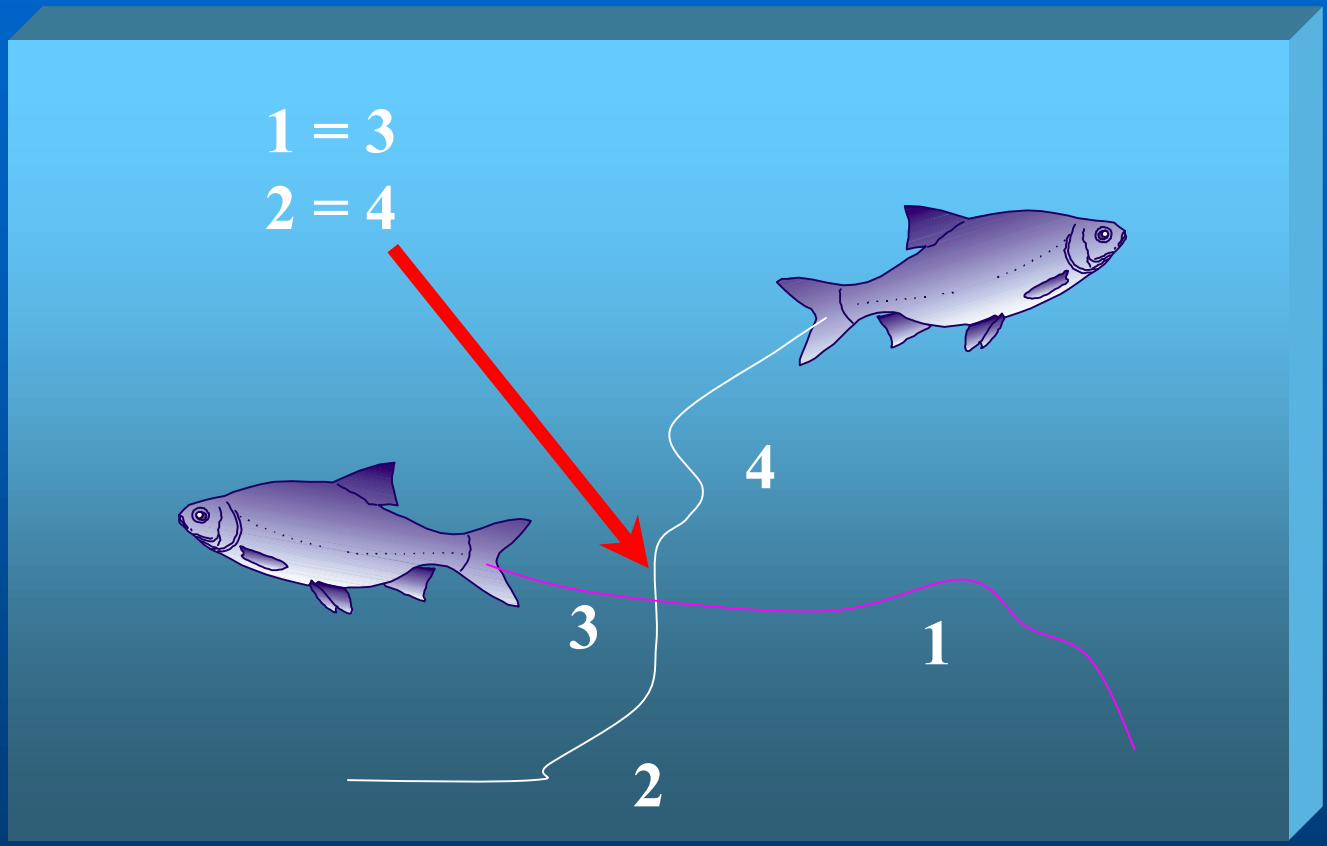
BehavioQuant - software and abilities (2)



Screenshot

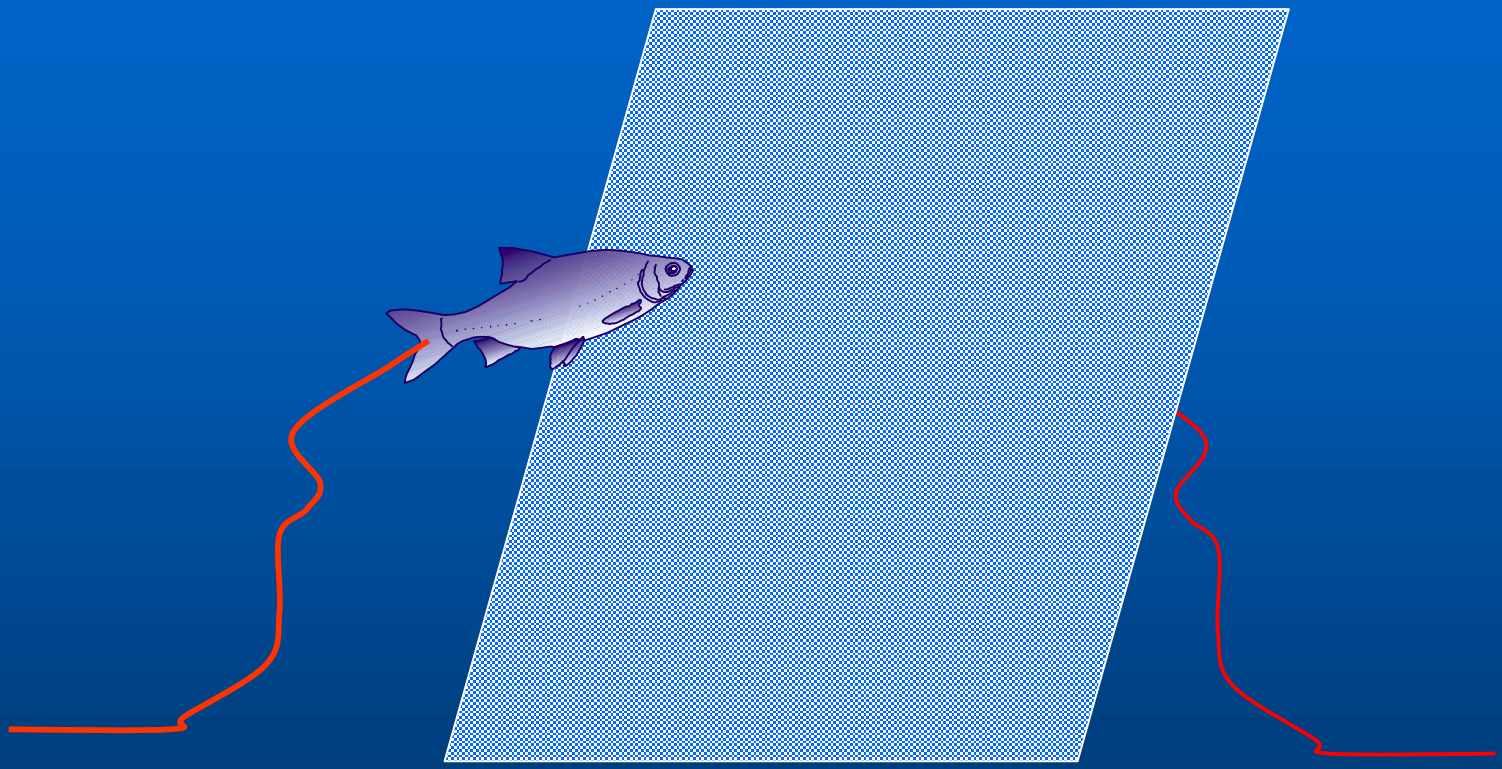
- Movement tracks of one fish group during one measuring interval
- Different lines represent single individuals
- 69 measuring cycles of 2 min per day, frequency of 25 frames per second

Problem 1 – crossing pathways



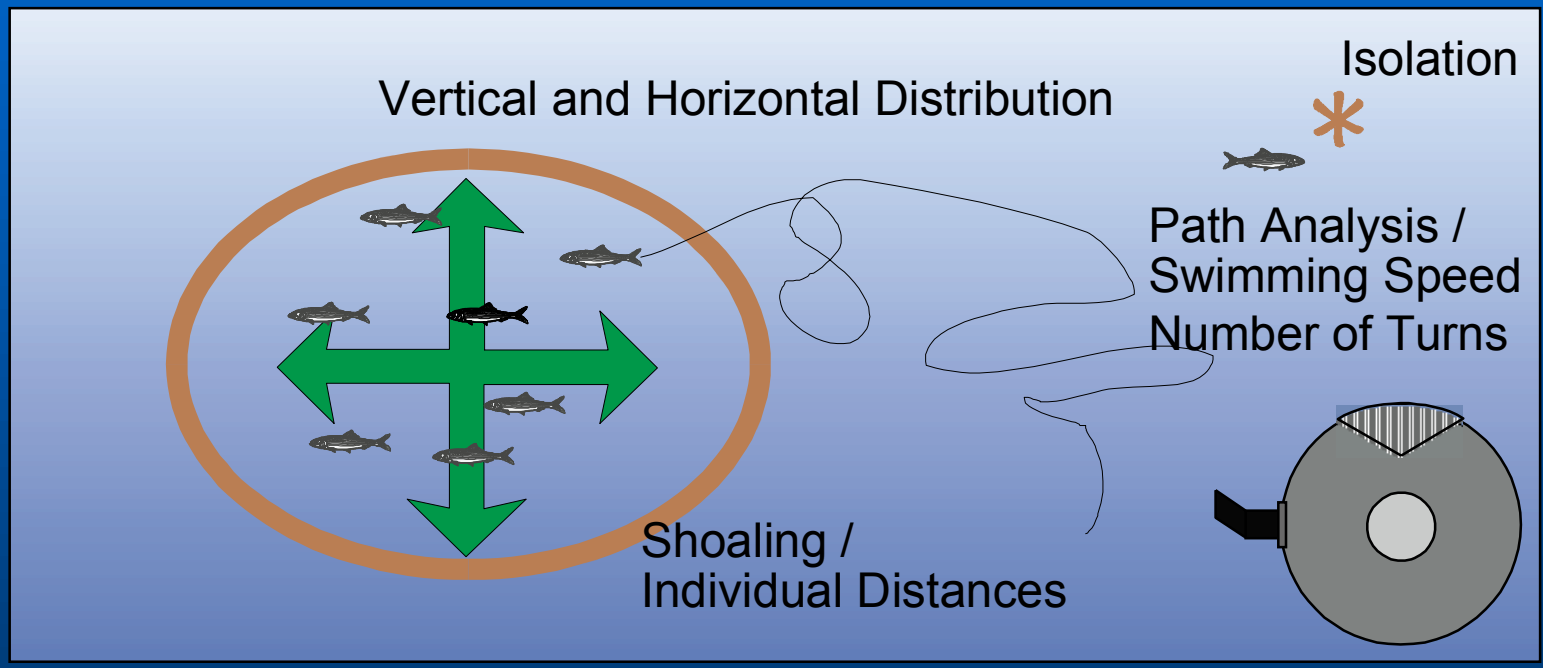
- Setting of a breakpoint in object-recognition algorithms
- All objects are given new numbers, new pathways are calculated
- Most similar objects are recombined

Problem 2 – mirror objects



- Mirror objects are effectively filtered by a time parameter
- They almost occur less than 40 % of the whole observation time

Recordable parameters of fish behaviour

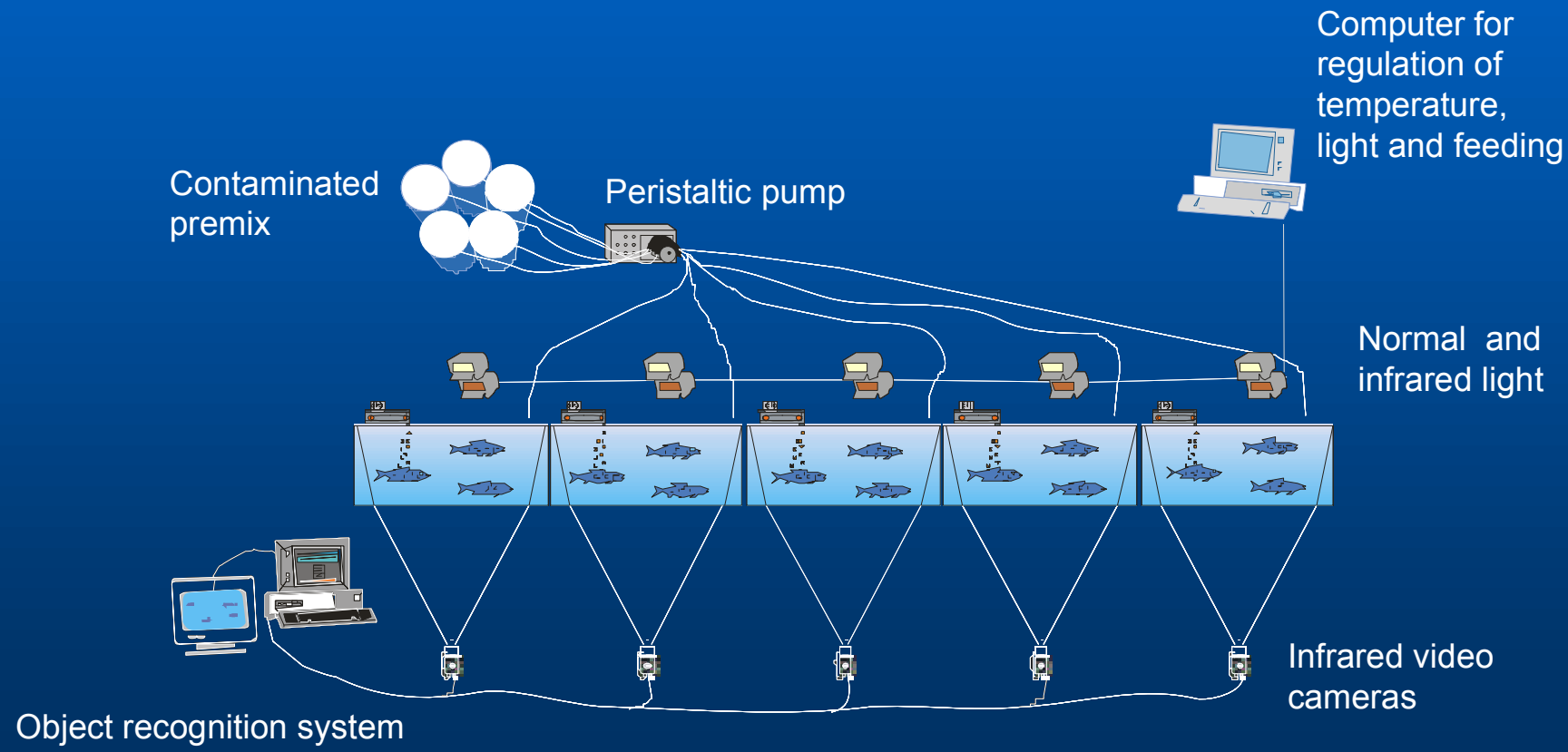


Results of behavioural video analysis

- *Experimental set-up*
- *Influences of MC-LR on general activity parameters*
- *Analysis and significance of activity rhythms*
- *Comparison of PCB 28 influences to MC-LR*

Experimental design

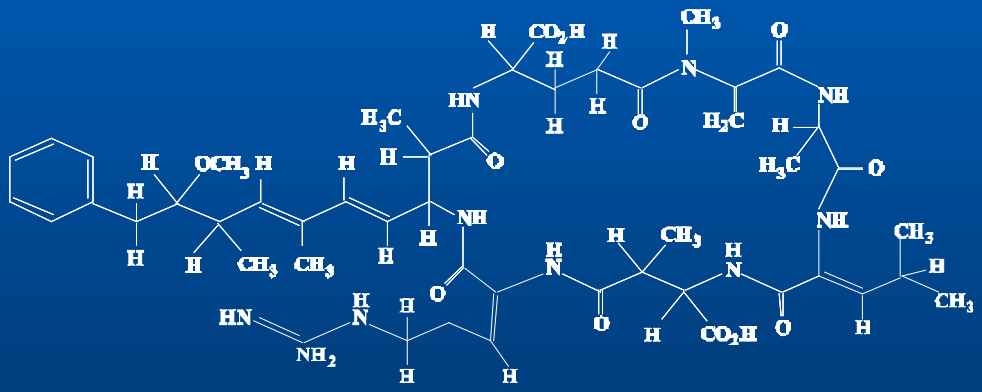
- 6 schools of seven adult individuals each were kept in 15-litre aquaria
- 3 weeks of acclimatisation
- Automated video processing system



Chemical stressors

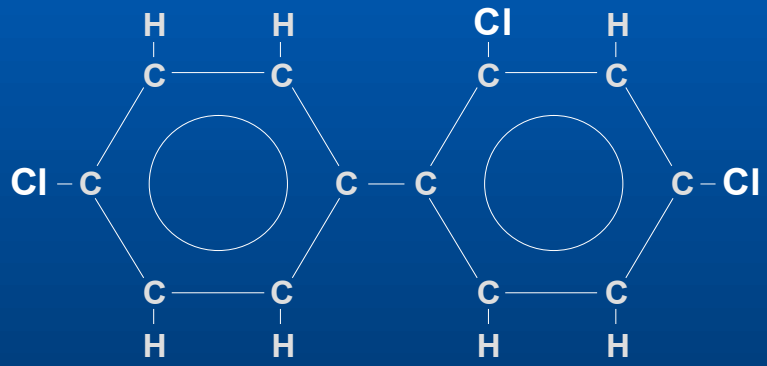
Microcystin-LR
(MC-LR)

- Natural Stressor



2,4,4`-Trichlorobiphenyl
(PCB 28)

- Anthropogenic Stressor

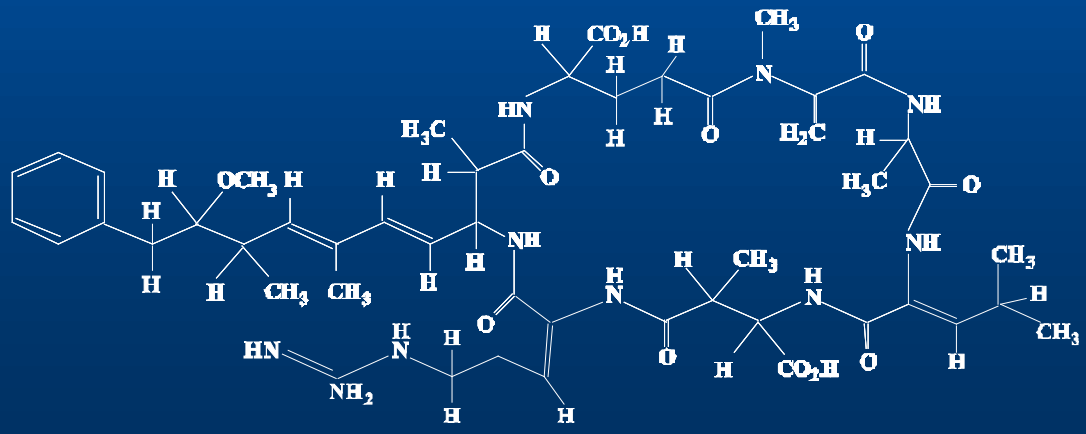


- Widespread in the aquatic environment
- Rather little knowledge about impact on fish behaviour

Cyanotoxin: Microcystin-LR (MC-LR)

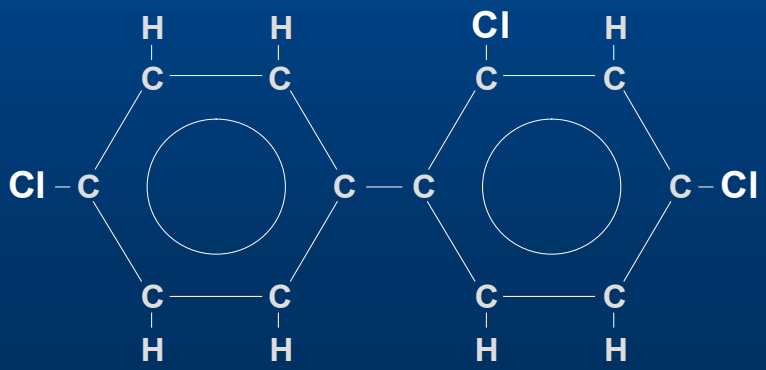
- Produced by the cyanobacterium *Microcystis aeruginosa* and other cyanobacteria
- Cyanobacteria blooms: up to 120 µg l^{-1} of dissolved MC-LR (Welker et al., 2001)
- Toxic effects of MC-LR on fish: e.g., damage to the liver, kidneys, or gills and mortality

  (Råbergh et al., 1991; Carbis et al., 1996; Fischer et al. 2000; Wiegand und Pflugmacher, 2005)



PCB 28: 2,4,4`-Trichlorobiphenyl ($C_{12}H_7Cl_3$)

- PCBs are chlorinated aromatic hydrocarbons
- Most of acute toxic effects on fish are related to commercial PCBs mixtures and to coplanar PCBs:
- Liver damage, impairment of osmoregulation and immune functions, endocrine effects and mortality
  (Hansen, et al., 1974; Monosson et al. 1994; Rice und Schenk, 1995; Zala und Penn, 2004)
- 2,4,4` trichlorobiphenyl: ortho-substituted PCB congener



Fish species

- Analysing potential species-specific reactions to chemical stressors

Danio rerio

(Zebrafish)

- Often used in toxical tests
- Tropical species

Leucaspius delineatus

(Sunbleak)

- Europe, Asia
- Temperate species

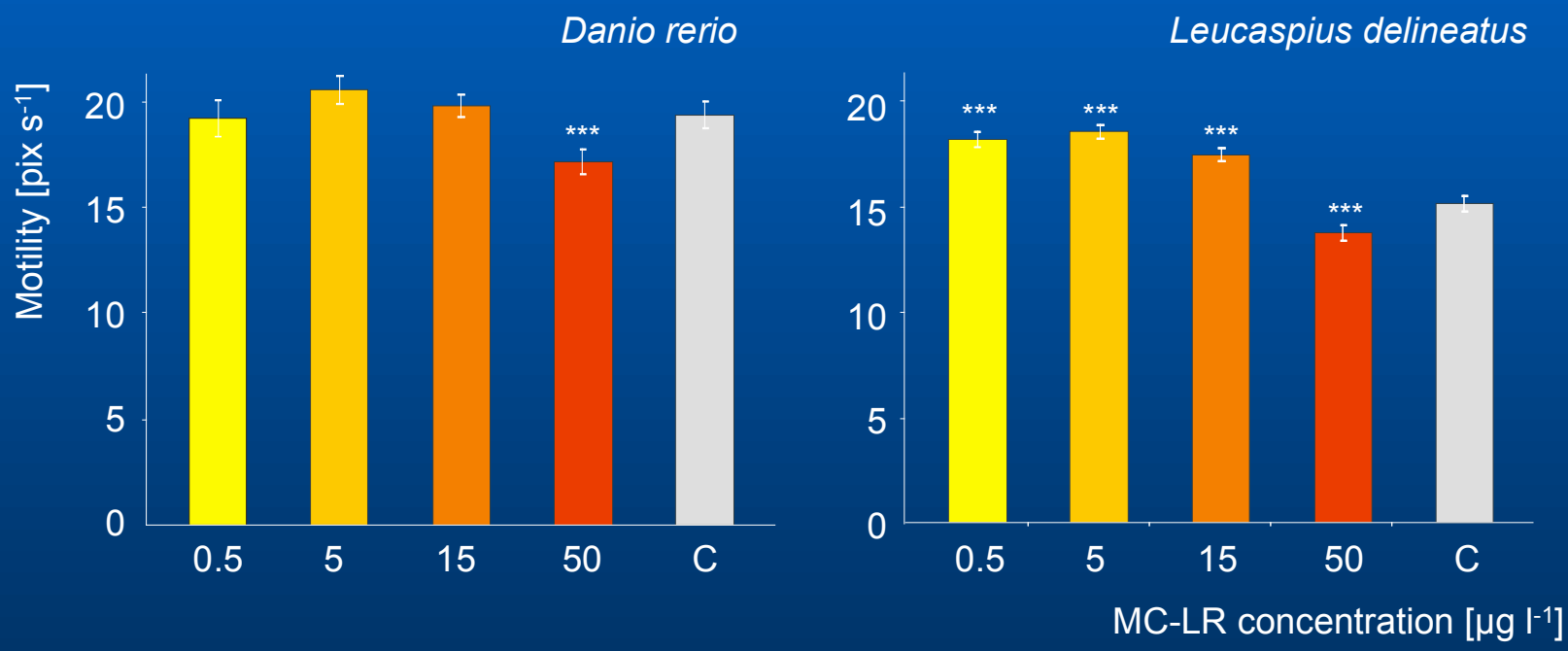
Motility

MC-LR

= swimming velocity in video-pixels per second

ANOVA, Dunnett T3 post hoc

* p< 0.05 ** p< 0.01 *** p< 0.005



Motility (Light / Dark)

MC-LR

Light phase Dark phase

ANOVA, Dunnett T3 post hoc

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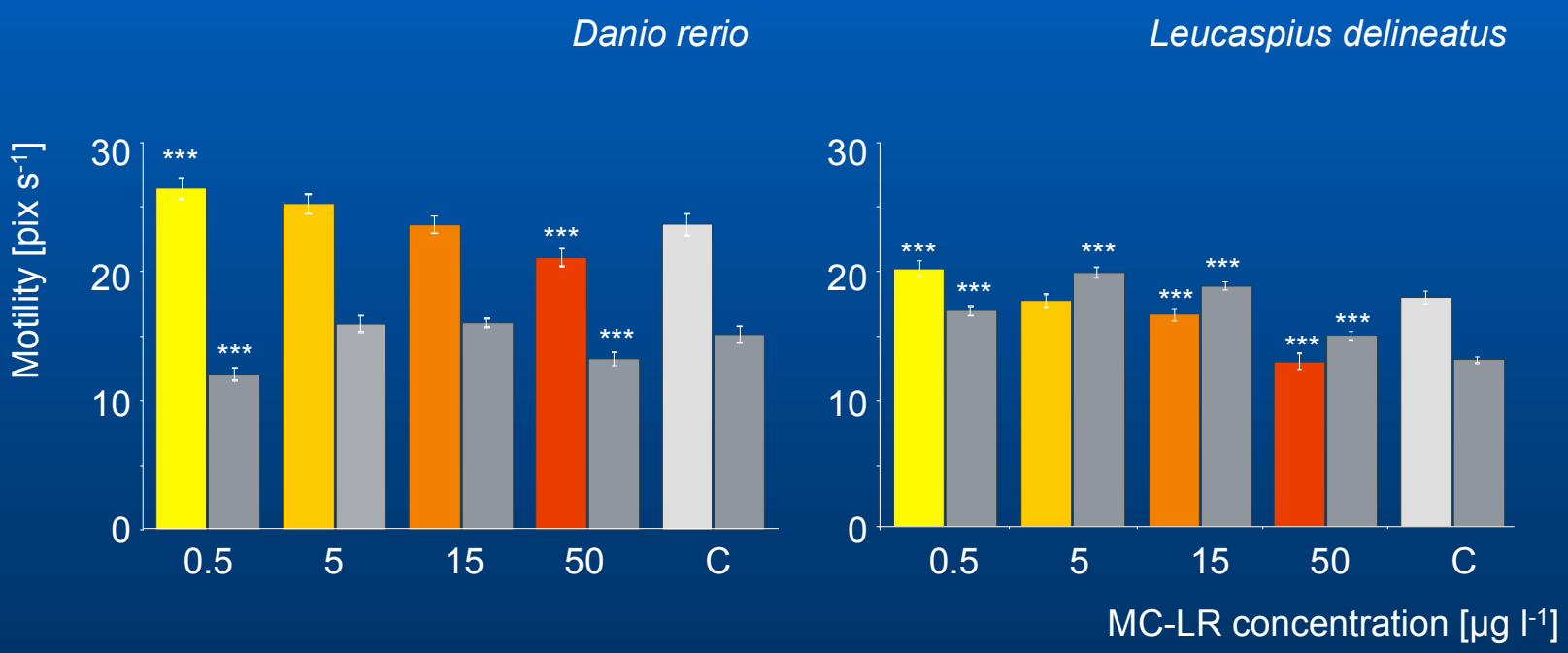

Turns

MC-LR

= number of changes of the direction per second

Light phase Dark phase

ANOVA, Dunnett T3 post hoc

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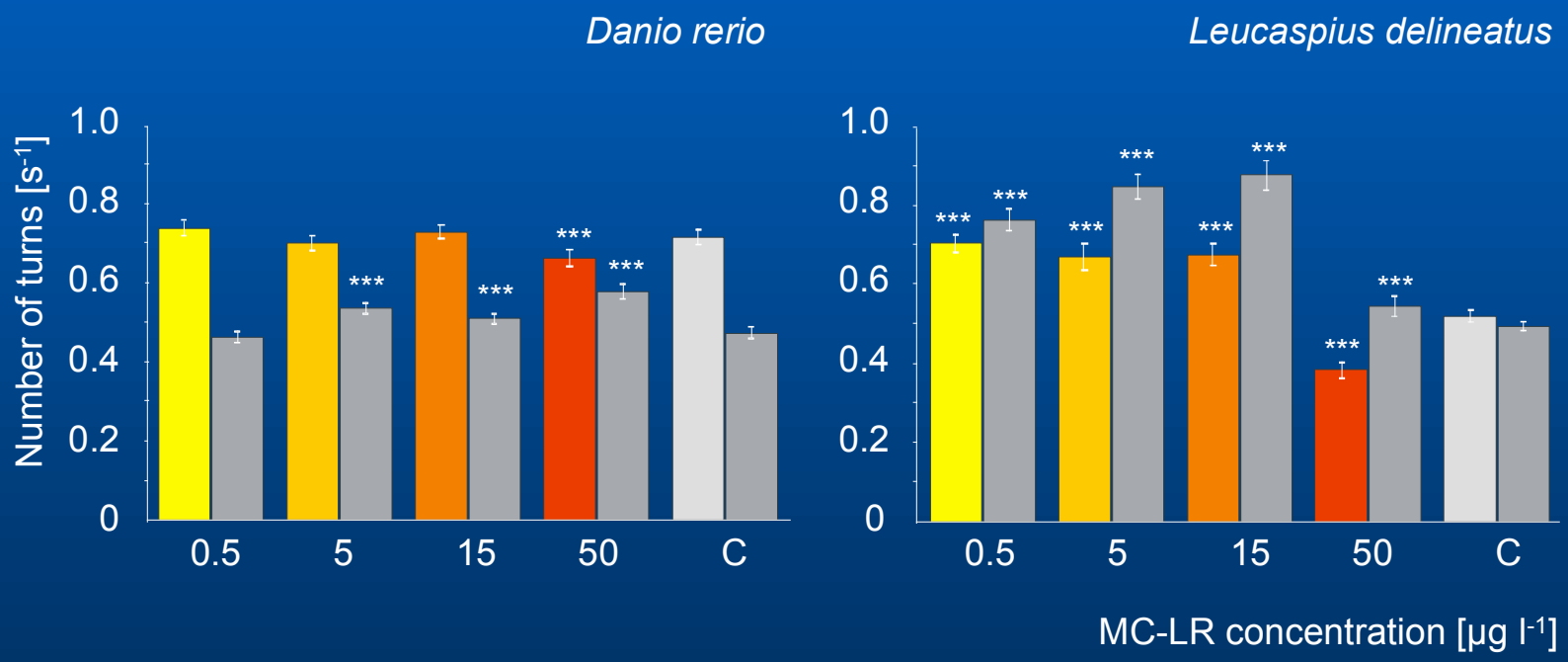
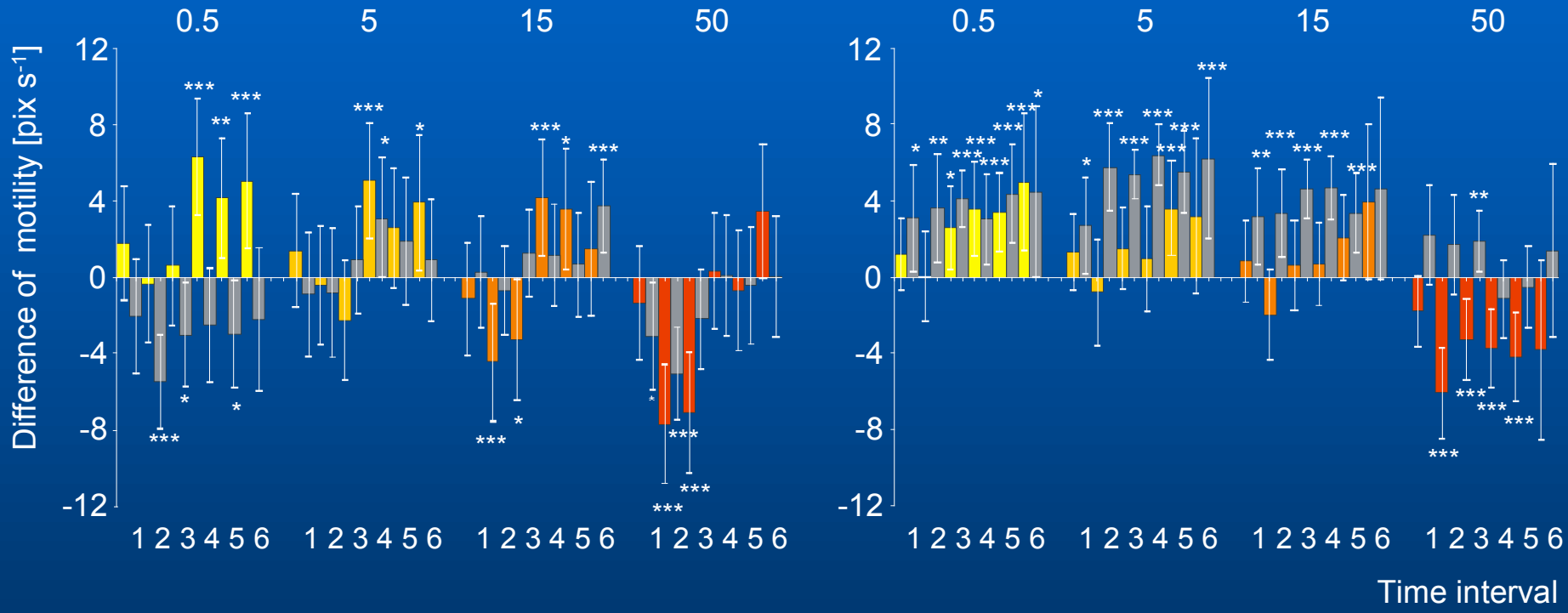


Temporal development of motilities MC-LR

Danio rerio *Leucaspius delineatus*

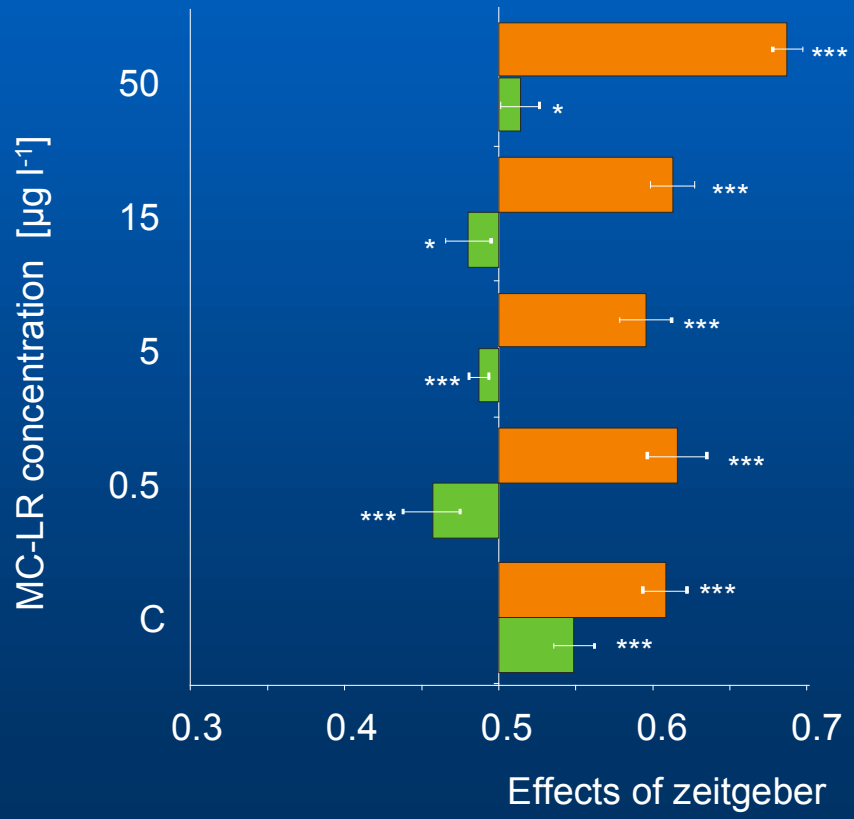


- 6 time intervals of exposure period (5 x 3 days und 1 x 2 days)

Light / Dark activity

MC-LR

$$\text{Effects of zeitgeber} = \frac{\text{motility of light phase}}{\text{overall motility (light and dark phases)}}$$



Student's *t*-test

* p< 0.05 ** p< 0.01 *** p< 0.005

Danio rerio

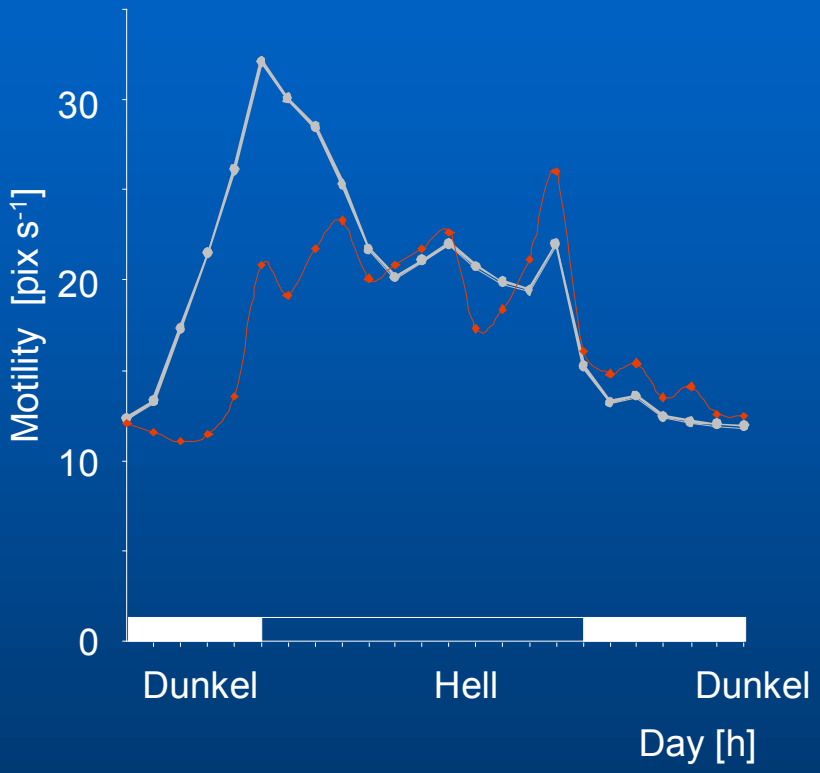
Leucaspius delineatus

Nocturnal active: 0.0 bis < 0.5

Diurnal active: > 0.5 bis 1.0

Effects of light on fish activity

MC-LR



Danio rerio

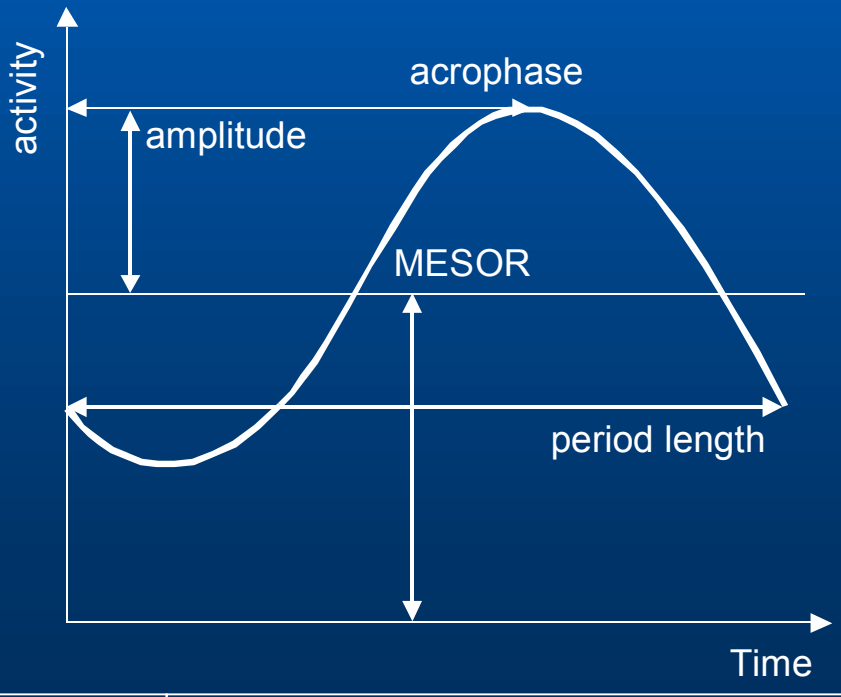
50 µg l^{-1}

Control

Reaction of fish to light on is reduced under chemical stress conditions

Time series analysis: 1. Cosine analysis

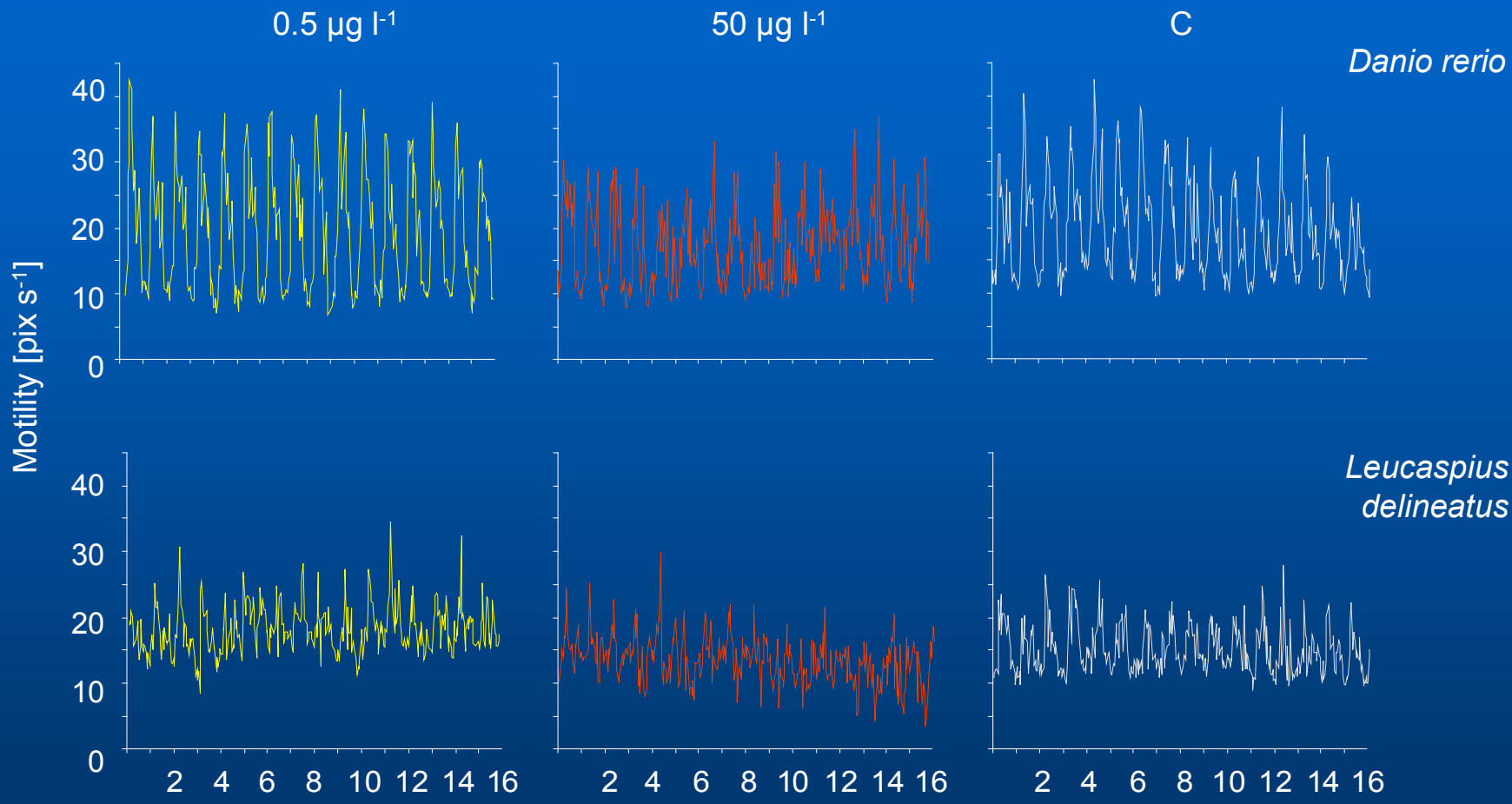
- Single cosine model using a non-linear regression procedure
- Applying a cosinus equation to the raw data series (SPSS 9.0)
- Using an approximation by sequential quadratic optimisation
- $f(x) = M + A * \cos (2p /P * (x - K))$



f(x)	observed data
x	given time data [hours]
M	MESOR or mean of the data series
A	amplitude (difference in the level between peak and through values)
P	period length [hours]
K	acrophase (location of peaks) [hours]

Cosine-Analysis

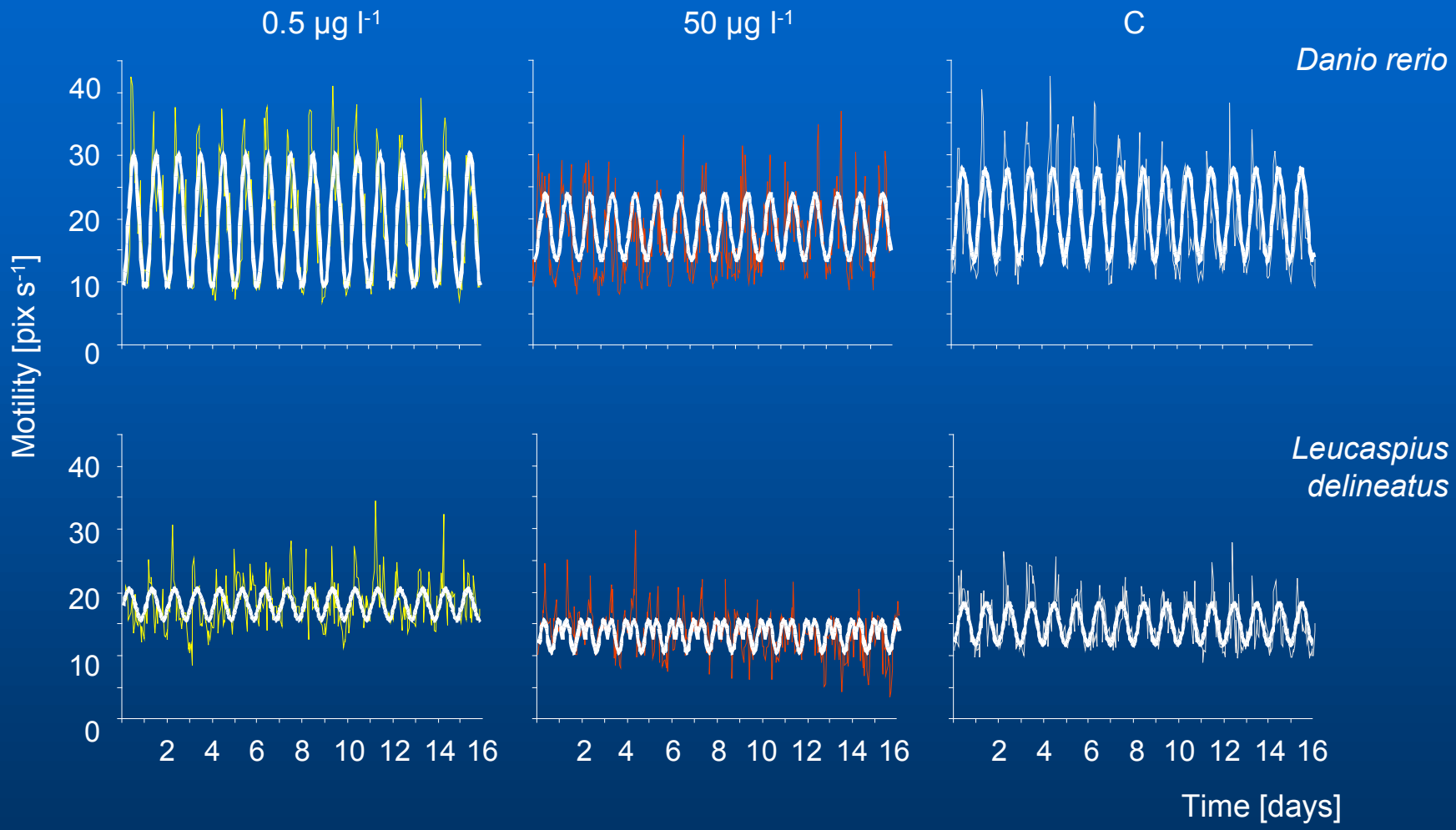
MC-LR



- Sequences of raw data over the whole exposure period

Cosine-Analysis

MC-LR



MESOR: mean of the data series – amplitude: difference in the level between peak and through values

Time series analysis: 2. Power spectral analysis

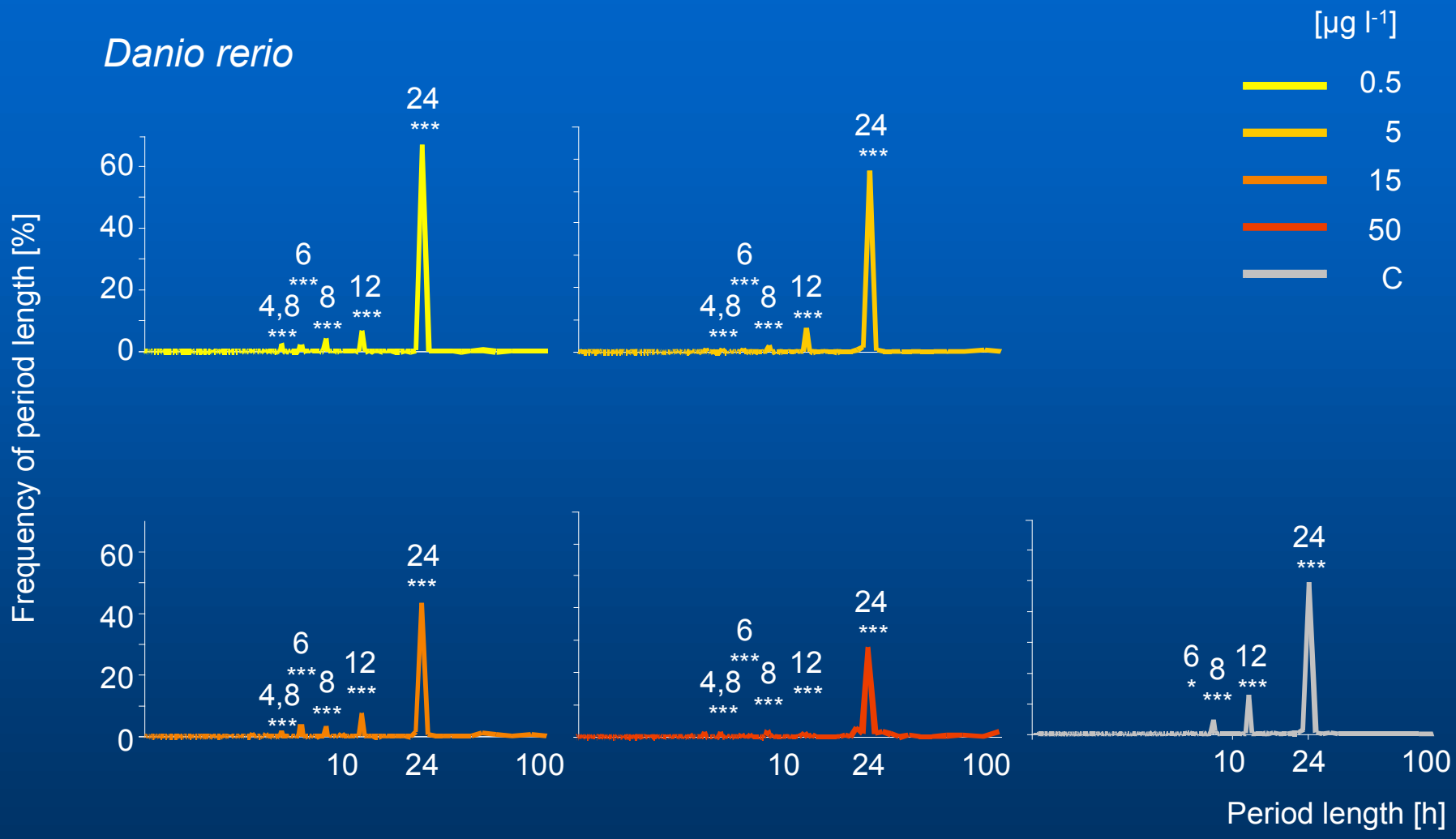
- Fourier transformed autocorrelation function
  Program “Zeit” (Scheibe et al., 1999, 2002)

- Quantification of the harmonic frequency structure of activity rhythms

- Data series are modelled with oscillations of different period lengths

Power spectral analysis

MC-LR



Quantification of the periodic frequency structure of activity rhythms

Motility

PCB 28

ANOVA, Dunnett T3 post hoc

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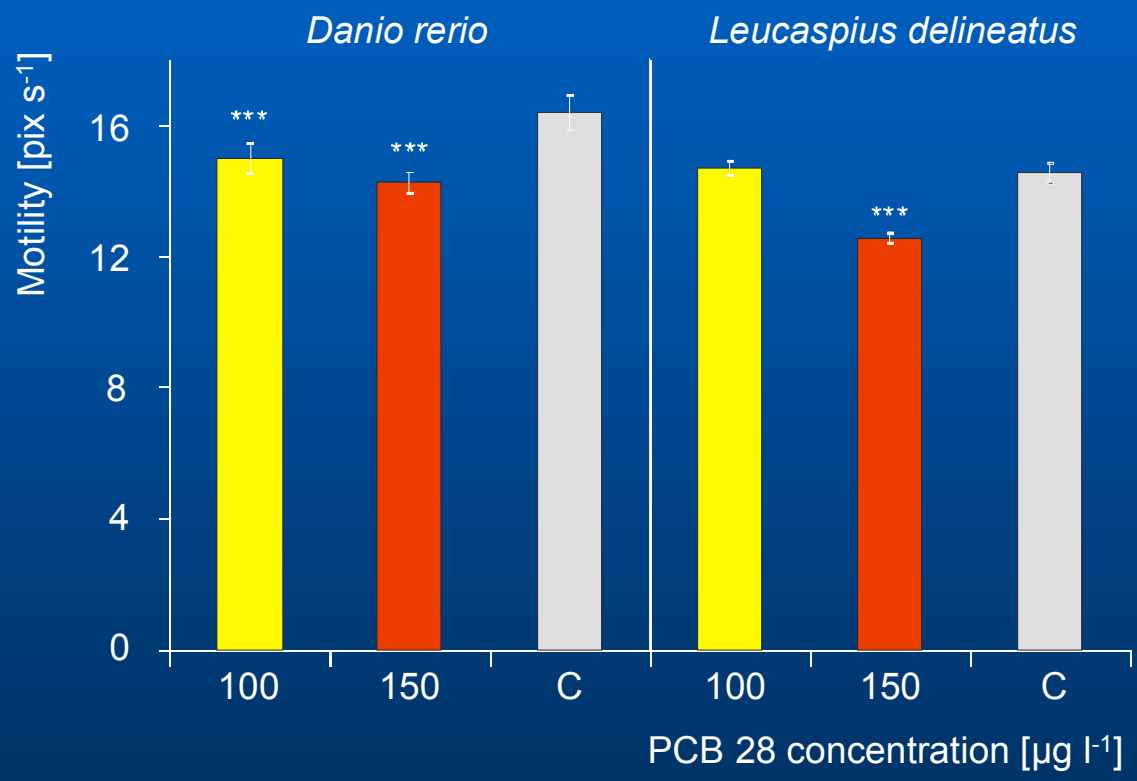

Motility (Light / Dark)

PCB 28

ANOVA, Dunnett T3 post hoc

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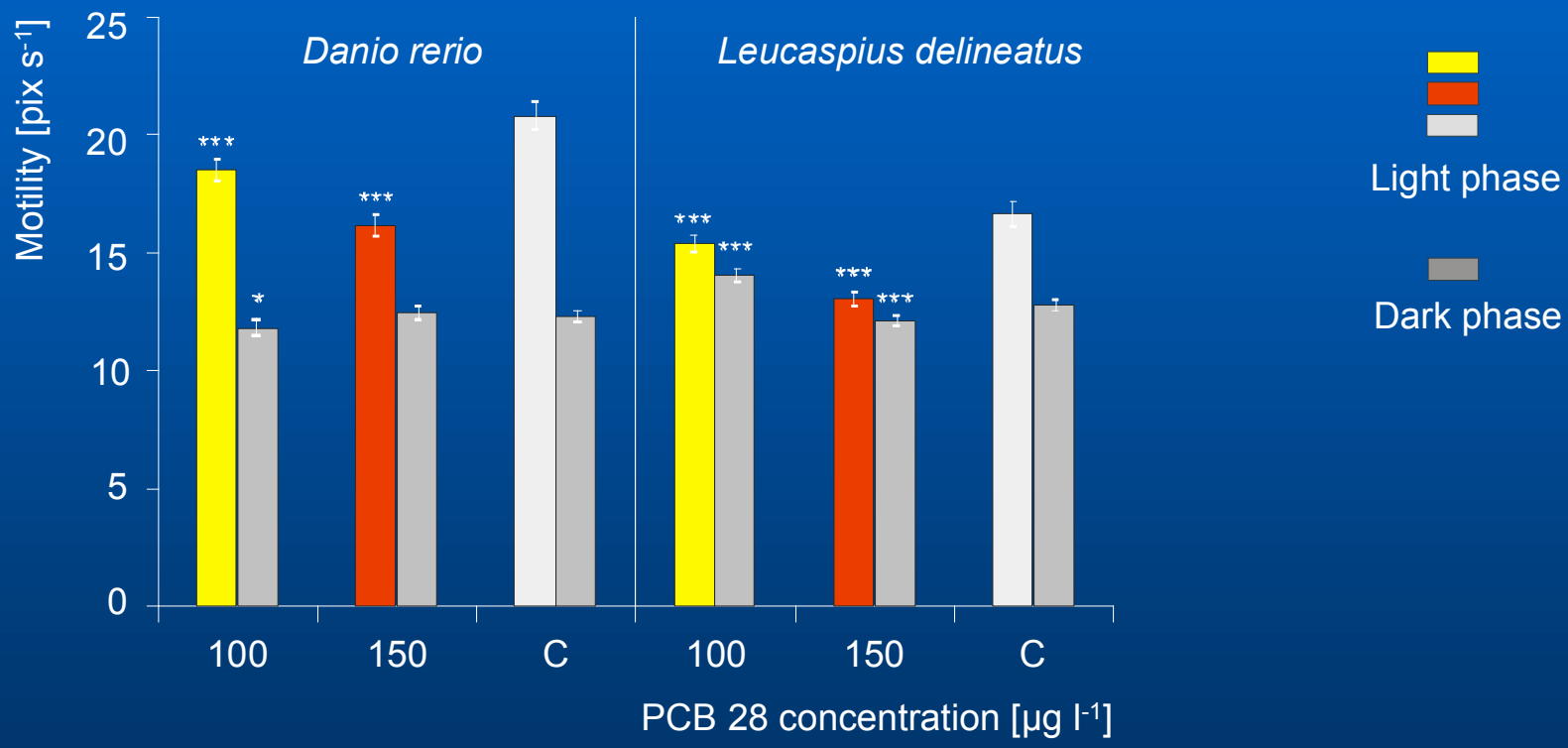
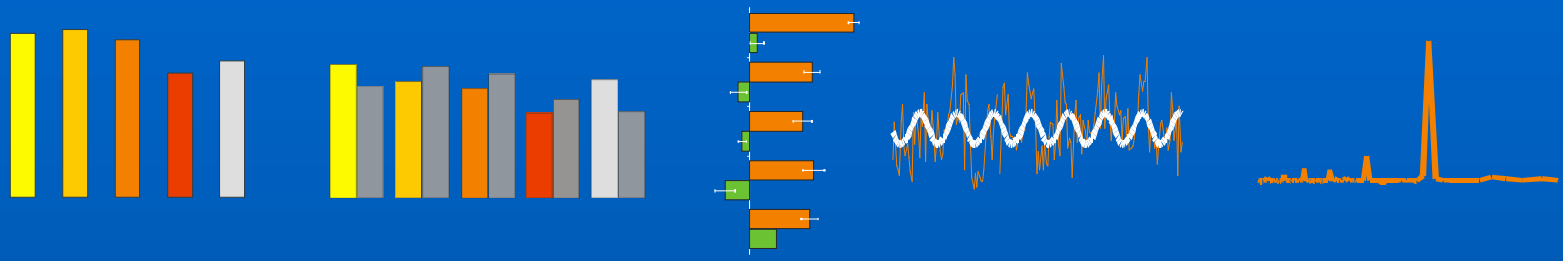
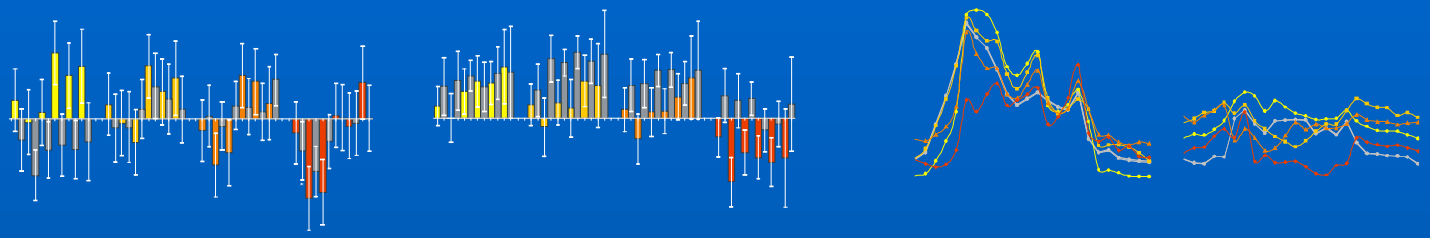


Summary



- Significant effects of both substances on the locomotor behaviour as well as on the rhythms of activity of both species
- Both stressors caused a significant decrease in activity
- Compensation effects between the diurnal and nocturnal phase of the day
- Use of chronobiological methods is helpful to increase the capability of behavioural studies

Conclusions for biomonitoring



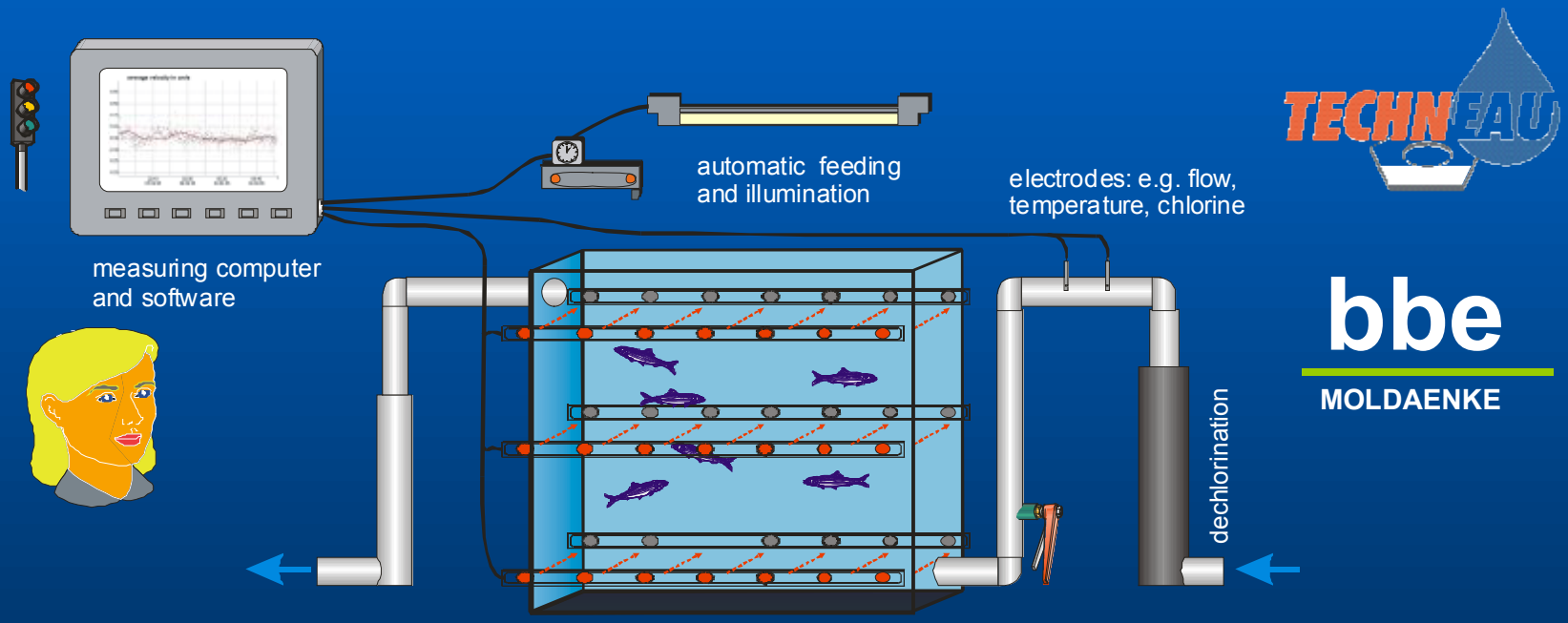
- Some of the standards of the experimental design of this study are relevant for biomonitoring
- Acclimatisation period and the constancy of external factors
- Usefulness to register the absolute deviation from standard values
- Several different patterns of animal reactions dependant e.g. on the species and the daytime

Thank you…

… for your attention.

Next stage of the work: *Tox*Protect 64

- Development of a low-budget biomonitoring system for drinking water protection (Project TECHNEAU)
- Aim: high reliability, indication of acutely dangerous situations

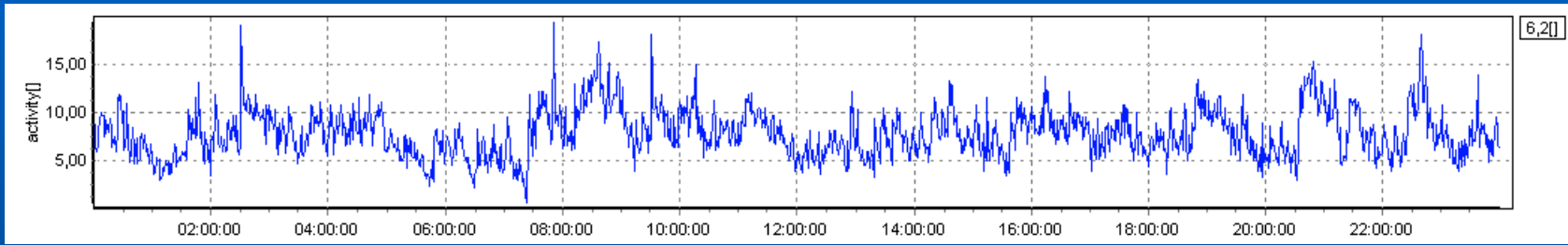


Monitor device: array of about 64 light barriers

Alarm triggers: software combines activity analysis with height distribution

Alarm Verification *Tox*Protect 64

- Natural random variations of fish behaviour
- Alarm criterion may be reached from time to time



- Integration of an alarm verification system to reduce false alarms
- Achieved by changing the illumination inside the aquarium
- Leads to a dramatic increase in activity
- Under toxic conditions this may not occur

→ Further laboratory tests